available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/peptides

Review

Hypothalamic LPXRF-amide peptides in vertebrates: Identification, localization and hypophysiotropic activity

Kazuyoshi Tsutsui [a,b,*], *Kazuyoshi Ukena* [a,b]

[a] *Laboratory of Brain Science, Faculty of Integrated Arts and Sciences, Hiroshima University, Higashi-Hiroshima 739-8521, Japan*
[b] *Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Corporation, Tokyo 150-0002, Japan*

ARTICLE INFO

Article history:
Received 12 March 2005
Accepted 22 June 2005
Published on line 3 March 2006

Keywords:
RF-amide peptides
LPXRF-amide peptides
Gonadotropin-inhibitory hormone (GnIH)
Frog growth hormone-releasing peptide (fGRP)
Hypothalamus
Pituitary
Hypophysiotropic activity

ABSTRACT

Probing undiscovered neuropeptides that play important roles in the regulation of pituitary function in vertebrates is essential for the progress of neuroendocrinology. Recently, we identified a novel hypothalamic neuropeptide with a C-terminal LPLRF-amide sequence in the quail brain. This avian neuropeptide was shown to be located in the hypothalamo–hypophysial system and to decrease gonadotropin release from cultured anterior pituitary. We, therefore, designated this novel neuropeptide as gonadotropin-inhibitory hormone (GnIH). We further identified novel hypothalamic neuropeptides closely related to GnIH in the brains of other vertebrates, such as mammals, amphibians, and fish. The identified neuropeptides possessed a LPXRF-amide (X = L or Q) motif at their C-termini. These LPXRF-amide peptides also were localized in the hypothalamus and other brainstem areas and regulated pituitary hormone release. Subsequently, cDNAs that encode LPXRF-amide peptides were characterized in vertebrate brains. In this review, we summarize the identification, localization, and hypophysiotropic activity of these newly identified hypothalamic LPXRF-amide peptides in vertebrates.

Contents

* *Corresponding author*. Tel.: +81 82 424 6571; fax: +81 82 424 0759.
E-mail address: tsutsui@hiroshima-u.ac.jp (K. Tsutsui).

0196-9781/$ – see front matter
doi:10.1016/j.peptides.2005.06.036

1. Introduction

Since the molluscan cardioexcitatory neuropeptide Phe–Met–Arg–Phe–NH_2 (FMRF-amide) was found in the ganglia of the venus clam [39], neuropeptides that possess the RF-amide motif at their C-termini (i.e., RF-amide peptides) have been characterized in various invertebrates [12,24,27–30,34,38,44,48,58]. The RF-amide peptides found in invertebrate nervous system can act as neurotransmitters and neuromodulators. These neuropeptides may also act through the endocrine system to control a variety of behavioral and physiological processes.

On the other hand, many immunohistochemical studies that used the antiserum against FMRF-amide suggested that vertebrate nervous systems possess some unknown neuropeptides similar to FMRF-amide [40,41]. In fact, neuropeptides that have the RF-amide motif at their C-termini have been identified in the brains of several vertebrates. First, Leu–Pro–Leu–Arg–Phe–NH_2 (LPLRF-amide), a chicken pentapeptide, was purified from the vertebrate brain [6]. Neuropeptide FF (NPFF) and neuropeptide AF (NPAF), two pain modulatory peptides [59], prolactin (PRL)-releasing peptide (PrRP) [13], and its fish counterpart *Carassius* RF-amide [10] are also RF-amide peptides. These RF-amide peptides may play some important physiological roles, such as neuroendocrine, behavioral, sensory, and autonomic functions. Among these vertebrate RF-amide peptides, NPFF is well documented as a morphine modulatory peptide [36,37,42].

In view of the modulatory action of morphine on neuroendocrine function [8] and our interest in avian reproductive neurobiology [22,23,49], we, therefore, looked for a novel avian RF-amide peptide that controls gonadotropin (GTH) and/or PRL secretion.

2. Isolation of novel hypothalamic LPXRF-amide peptides

To isolate the RF-amide peptide from the brain, Japanese quails (*Coturnix japonica*) were used and the peptidergic molecule was probed with competitive enzyme-linked immunosorbent assay (ELISA), employing the antibody against the dipeptide, Arg–Phe–NH_2 [50]. A novel peptide was isolated and shown to have the sequence SIKPSAYLPLRF-amide (Table 1) [50]. This neuropeptide had not been previously reported in vertebrates, although the C-terminal LPLRF-amide was identical to chicken pentapeptide LPLRF-amide peptide [6]. The chicken peptide may be a degraded fragment of the dodecapeptide, because Dockray et al. [7] have speculated that a longer peptide may be present as a mature peptide in the chicken brain. Subsequently, the isolated novel peptide was shown to be located in the quail hypothalamo–hypophysial system and to decrease gonadotropin release from cultured anterior pituitary in a dose-dependent manner [50]. We, therefore, designated this novel RF-amide peptide as gonadotropin-inhibitory hormone (GnIH) [50].

After the isolation of GnIH, we further sought to identify similar RF-amide peptides in other vertebrate classes that regulate secretion of hormones by the anterior pituitary gland [20]. We first turned to amphibia. We identified in the bullfrog a novel hypothalamic RF-amide peptide (SLKPAANLPLRF-amide; Table 1) [20]. Although this frog RF-amide peptide had not been previously reported to exist in vertebrates, it was revealed to have a high sequence homology (75%) with quail GnIH (Table 1). In addition, the frog peptide possessed growth hormone (GH)-releasing activity, and was designated as frog GH-releasing peptide (fGRP) [20]. Collectively, these results show that the novel RF-amide peptides, GnIH and fGRP, possess the same C-terminal motif, LPLRF-amide [20,50].

At the same time as we reported the identification of fGRP, two other research groups independently isolated the same peptide as fGRP and its related peptide from European green frog and bullfrog, respectively. Chartrel et al. [5] isolated an RF-amide peptide from the brain of European green frog with a radioimmunoassay for bovine NPFF, and designated *Rana* RF-amide (R-RFa). This R-RFa is structurally identical to fGRP purified from the bullfrog hypothalamus (Table 1) [5,20]. On the other hand, Kanetoh et al. [16] isolated a different RF-amide peptide (SIPNLPQRF-amide) from the bullfrog brain, using the dot immunoblot analysis with an antiserum to FMRF-amide (Table 1). Although this RF-amide peptide was named frog nociception-related peptide (fNRP), it is identical to fGRP gene-related peptide-1 (fGRP-RP-1), which is encoded in the cDNA of fGRP precursor polypeptide (see next chapter).

Turning to mammals, cDNAs that encode novel RF-amide peptides similar to GnIH and fGRP have been detected in mammalian brains with a gene database search [14]. The cDNAs of human and bovine peptides encoded three peptides,

Table 1 – Amino acid sequences of the identified LPXRF-amide peptides in vertebrate brains

Sequence	Animal	Name	Reference
SLTFEEVKDWAPKIKMNKPVVNKMPPSAANLPLRF-NH_2	Bovine	RFRP-1	[11]
AMAHLPLRLGKNREDSLSRWVPNLPQRF-NH_2	Bovine	RFRP-3	[62]
ANMEAGTMSHFPSLPQRF-NH_2	Rat	RFRP-3	[54]
SIKPSAYLPLRF-NH_2	Quail	GnIH	[50]
SSIQSLLNLPQRF-NH_2	Quail	GnIH-RP-2	[45]
SLKPAANLPLRF-NH_2	Bullfrog/green frog	fGRP/R-RFa	[5,20]
SIPNLPQRF-NH_2	Bullfrog	fGRP-RP-1/fNRP	[16,55]
YLSGKTKVQSMANLPQRF-NH_2	Bullfrog	fGRP-RP-2	[55]
AQYTNHFVHSLDTLPLRF-NH_2	Bullfrog	fGRP-RP-3	[55]
SGTGLSATLPQRF-NH_2	Goldfish	gfLPXRFa-3	[46]

LPXRFamide (X = L or Q) motif is underlined.